**IN-SOLUTION SAMPLE DIGESTION PROTOCOL**

REAGENTS REQUIRED

1. Ammonium bicarbonate (Sigma A6141) - working concentration 50 mM

2. Dithiothreitol (Sigma D5545) - working concentration 100 mM

3. Iodoacetamide (Sigma I1149) - working concentration 200 mM

4. MS grade Trypsin (Sigma T6567) - working concentration 0.4 µg /µl

5. Formic acid (Merck 5.33002.0050)

REAGENT SETUP (prepare freshly before use)

1. DTT solution: 100 mM DTT in 50 mM ammonium bicarbonate.

2. Iodoacetamide solution: 200 mM Iodoacetamide solution in 50 mM ammonium bicarbonate.

3 Formic acid 1% in milliQ water (v/v).

4. Trypsin buffer: 0.4 µg /µl trypsin in 50 mM ammonium bicarbonate.

Trypsin digestion protocol for 100 µg of protein sample

1. Add 5 µl of 100 mM DTT solution to the protein sample.

2. Incubate at 60º Celsius for 30 minutes.

3. Add 5 µl of 200 mM Iodoacetamide solution to the protein sample.

4. Incubate at room temperature in dark for 30 minutes.

5. Add trypsin buffer in the ratio of 1:25 (1µg of trypsin to 25µg of protein).

6. Incubate at 37º Celsius overnight with gentle shaking.

7. To arrest the reaction, add 1 µl of 1% formic acid (v/v) to the samples.

8. Incubate at 37º Celsius for 20 minutes.

9. Store the samples at -20 deep freezer until use.